Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone

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Summary

1) In this review, we describe the hierarchical interplay of hydrology, hyporheic ecology and transformation of nutrients and pollutants in the hyporheic zone (HZ). The interchange of water between the surface-subsurface generates the hyporheic hydrodynamics: the engine that drives the ecological functioning of the HZ. The magnitude and direction of hydrological fluxes in the HZ follow complex spatial patterns, strongly influenced by the temporal dynamics of surface flow in rivers.

2) The direction and magnitude of hydrological fluxes also shapes the structure of hyporheic communities (hyporheos). During surface disturbances such as flooding or drought, benthic organisms may also use the HZ as a refuge, although the importance of this role is debated.

3) Streambed organisms differ in their ability to colonise the HZ depending on the biological traits they possess. The reduction in oxygen concentration and pore size with increasing depth imposes a limit on the distribution of macroinvertebrates, which are replaced by a suite of smaller organisms (meiofauna and protists) at deeper sediment layers. Therefore, a concomitant reduction in net biomass and productivity might be expected along a vertical gradient. However, only a few studies have assessed the contribution of the hyporheos to whole system production, and they have focused only on the fraction of relatively large organisms.

4) The bioreactor ability of the HZ to transform nutrients and pollutants is an important ecosystem service sustained by the life activities of hyporheos. Biofilms have the key role in this process due to their capacity to metabolize a wide range of dissolved

compounds, including emerging pollutants. However, the residence time of water in pore sediments (resulting from hyporheic hydrodynamics) and the rest of the community (constantly reworking the sediments and grazing biofilms) are indirectly involved.

5) In order to understand how hyporheic hydrodynamics may determine the ecology of hyporheos, and its implications for the bioreactor functioning of HZ, future research in this system should combine modern and truly interdisciplinary approaches at the interface of hydrology, geomorphology and ecology.

Key words: macroinvertebrates, meiofauna, biofilms, micropollutants, bioremediation, ecosystem services.

Introduction

In most lotic systems, the surface water of the open channel is connected to groundwater systems via the riverbed sediments. As a result, there is a bi–directional exchange between the groundwater and the surface along the continuum of stream and rivers (Bencala 1993). The volume of sediments in which stream water mixes with groundwater is known as the hyporheic zone (HZ). HZ functioning in the context of the whole–river ecosystem has been studied by researchers belonging to many different disciplines and as a result selecting a single inclusive definition for the HZ is difficult (Bencala 2000). Traditionally its definition has depended on the discipline–specific interest in hyporheic processes (Tonina & Buffington, 2009; Table 1). For example, in Geochemistry, the HZ is defined as the volume of sediment containing a specified percentage of surface water, while in Biology it is described as the volume of sediments housing a characteristic hyporheic community. Even the vertical extent of the HZ is not unanimously agreed. These differences in definition and extent have important implications for study methodology. However, the fundamental concept behind all definitions is that water exchanges between the open channel and the ground water systems.

Recently, Ward (2016) proposed a more flexible and cross–disciplinary definition (Table 1). A key idea from this definition of HZ is the importance of the temporal scale relevant to the processes of interest. In fact, flow paths and the rates of water exchange through the HZ are strongly influenced by the temporal dynamics of surface flow in rivers. This is especially evident on a seasonal scale. Despite the constant dynamism to which rivers are subjected, seasonality may result in a set of drastic changes in water flow conditions

79 (Gasith & Resh 1999) and determine the location and extent of HZ (Wondzell 1993).
80 Nonetheless, the HZ buffers the amplitude of this variation, acting as a potential refuge of
81 riverbed biota during adverse conditions (*See* Stubbington 2012). This has important
82 implications for variation in the composition and abundance of organisms throughout the year
83 (Stubbington et al. 2009). The HZ harbours diverse and productive communities whose
84 distribution and composition is strongly correlated with the direction and magnitude of
85 hydrologic fluxes (Stanley and Boulton 1993, Olsen and Townsend 2003). These hyporheic
86 communities or hyporheos (*hypo* = under, *rheos* = river) are composed of microbial biofilms
87 (bacteria and fungi existing in an exocellular matrix, Singer et al. 2006), protists (mainly
88 ciliates, flagellates and amoebae) and invertebrates. These groups differ notably in their
89 biological traits and ability to colonize the riverbed, shaping the budget of biomass and
90 secondary production in the HZ.

91 The HZ is a mechanical filter mediated by the pore space of sediments and water
92 flows, a biochemical filter controlled by biological and chemical processes, and a photic filter
93 (Boulton et al. 2010). As a result, the HZ provides an important ecosystem service acting as a
94 true bioreactor (hyporheic bioreactor, Table 1) with an impressive self–purification capacity
95 in lotic systems, and a barrier against contamination of aquifers, which are essential in the
96 supply of water for human consumption (Lewandowski et al. 2011). Thus the HZ of streams
97 and rivers has a critical role in the flows of biomass and energy, cycling of nutrients and
98 pollution attenuation (McClain et al. 2003, Smith et al. 2009, Robertson & Wood 2010,
99 Boulton et al. 2010). A large body of literature describes the nitrogen, phosphorus and
100 organic carbon attenuation in the HZ of streams and rivers (i.e. Harvey et al. 2013, Aubeneau
101 et al. 2015, Stegen et al. 2016, Liu et al. 2017). However, there are few data assessing the

fate and removal rates of the emerging micropollutants (Table 1) in lotic systems (Lewandowski et al. 2011, Köhler & Triebskorn, 2013), making understanding about the processing of these compounds by the bioreactor a remaining challenge in ecology.

The role of the hyporheic bioreactor in the whole river system might be seen, in an analogous way, as the 'rivers liver' (Fischer et al. 2005). HZ has an important role on the production, metabolisms, interchange and transformation of dissolved compounds, and health of the whole ecosystem. Here we describe the hierarchical relationship between hyporheic hydrodynamics, community ecology, and pollutant attenuation of the HZ. These subjects have been mainly assessed separately in discipline specific studies but they are intimately connected and together drive the functioning of the hyporheic bioreactor.

Hyporheic hydrodynamics: the motor of hyporheic zone ecology

The water interchange between the open river channel and a groundwater system generates the hyporheic hydrodynamics and strongly influences the whole ecosystem by determining the transport of solutes between compartments (Ward et al. 2012) and the chemistry of stream water (Duff and Triska 1990, Bencala et al. 1993). Streambed sediments are a porous medium through which exchange of water occurs. Thus, the hyporheic exchange and the flow paths throughout sediments could be theoretically studied by applying Darcy's Law, as the product of hydraulic gradient and hydraulic conductivity values (Jones et al. 1996). However, there are a wide range of additional factors, such as the composition of sediment and the rate of mixing that act at different scales and also influence hyporheic hydrodynamics (Larkin & Sharp 1992, Hakenkamp et al. 1993, Boulton et al. 1998,

124 Buffington & Tonina 2009). Therefore, hyporheic hydrodynamics do not show a uniform
125 pattern along the river. The flow between surface and groundwater follows complex
126 dynamics (Rutheford & Hynes 1987), in which hydrologic up–welling (UW) and
127 downwelling (DW) zones occur alternately (White 1990). Thus, as a result of this mosaic of
128 physical features, sediment conditions may change substantially even at a centimetres scale
129 (Boano et al. 2014). In addition, temporal dynamics from daily to seasonal processes result in
130 re–adjustments in hyporheic flow through time (Wondzell 2011).

131 Streambed topography from sediment–scale (little dunes or pebbles) to larger
132 geomorphologic features (i.e., riffle–pool sequence, steps), is the primary control on
133 hyporheic exchange (Maddock et al. 1995, Vallet et al. 1996, Dahm et al. 1998, Calver
134 2001,Ward et al. 2012, Gomez–Velez et al. 2014). These features act as obstacles to the water
135 flow along the open channel, extending the HZ both vertically and laterally from the stream
136 (Harvey & Bencala, 1993), and generating sequences of DW and UW zones (Savant et al.
137 1987, Hendriks & White, 1991, Harvey & Bencala 1993), for example, within a single stream
138 riffle, surface water enters the HZ at the beginning of the riffle (DW zone) and returns to the
139 open channel at the end of it (Hendrik & White 1991, Hendriks 1993, Evans & Petts 1997).

140 Sediment grain size is another factor influencing hyporheic exchange (see Table 2 for
141 a definition of terms). This is generally faster in headwater streams, which typically have
142 shallow and steep stretches with cobble– and gravel–bed sediments (more porous). Hyporheic
143 exchange is progressively slower, deeper and more complex as riverbed sediments become
144 finer (less porous) in slower flow zones (Buffington & Tonina 2009). Nevertheless,
145 quantifying this exchange is complicated because streambed materials range from relatively

146 homogeneous, to cases where the range of sediment sizes (and therefore the hydraulic
147 conductivity) exceeds six orders of magnitude (Calver et al. 2001).

148 In recent years a wide range of available sampling techniques have been developed to
149 determine the heterogeneous interactions between ground water and surface water (Kalbus et
150 al. 2006). These methods range from direct measurements of water flux across the
151 groundwater–surface water interface (i.e. seepage meter, Lee 1977), to indirect techniques
152 such as heat tracer methods, mass balance approaches or mathematical modelling (Kalbus et
153 al. 2006). Until recently these were almost exclusively employed in the field of hydrology
154 and engineering (Boulton et al. 2010). However, understanding the importance of hyporheic
155 hydrodynamics as a controlling factor of hyporheic communities and biochemical processes
156 has led to increased implementation of hydrodynamic analysis in recent ecological studies of
157 the HZ (Boulton 1993, Hendricks 1993, Standley and Boulton 1993, Schmid–Araya 1998,
158 Kasahara et al. 2009, Miyake & Shigeru 2002, Malard et al. 2003, Davy–Bowker et al. 2006,
159 Robertson & Wood 2010). One interesting strategy to address these issues, which has been
160 widely used in hydrological studies, is to implement time–series analysis of streambed
161 profiling–thermal records (i.e. Hatch et al. 2006, Keery et al. 2007, Irvine & Lautz 2015,
162 Irvine et al. 2015). These methods are based on quantifying changes in phase and amplitude
163 of temperature variations between pairs of subsurface sensors through depth (Hatch et al.
164 2006). Nevertheless, results exclusively from these methods are often limited and
165 contradictory (Shanafield et al. 2011, Briggs et al. 2014).

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167 Seasonality in flow exchange and its effect on riverbed communities

168 Stream and rivers are dynamic ecosystems par excellence. The incessant open channel flow
169 produces continual movements of the substrata, changes to the streambed topography and
170 reorganization of the channel boundaries morphology. In this manner, the temporal dynamics
171 of surface flow in a lotic ecosystem is closely related to the spatial heterogeneity of flow
172 paths and rate of exchange through the HZ at all scales. The multiple temporal and spatial
173 scales and the rate of exchange collectively define the hyporheic residence time of water
174 (Buffington & Tonina 2009). The residence time is an important property of the HZ, because
175 most of the biogeochemical processes that occur in sediments depend on the rate of water
176 flow through them (Mulholland & De Angelis 2000, Duff & Triska 2000). Furthermore, the
177 temporal dynamic that affects the hydrological exchange also produces fluctuations of HZ
178 boundaries (Gibert et al. 1990) and this variation of the HZ size determines its influence on
179 both the surface open channel and the underlying groundwater (Vervier et al. 1992).

180 Seasonality in rivers is an extreme example of the temporal variation of the open
181 channel discharge (e.g. due to snowmelt or the alternation between dry and rainy seasons).
182 Accordingly, these changes alter the flow exchange patterns in the HZ (Kalbus et al. 2006)
183 and may act as disturbances for sediment organisms (Robertson et al. 1995, Townsend et al.
184 1997, Robertson 2000). These potential disturbances may be reduced in the HZ due to its
185 ability to maintain humidity after surface drying and remain stable during floods (Boulton et
186 al. 1998). Thus the HZ might serve as a refuge for the local biota during disturbances events
187 enabling recolonization of the surface once the disturbance ends (Williams & Hynes 1974,
188 Dole–Olivier 2011). The HZ can also act a refuge for the early instars of some
189 macroinvertebrates due to the more stable environmental conditions and reduced predator
190 pressure (Williams 1984). Nevertheless, the importance of the HZ as a refuge is debated

191 (Robertson & Wood 2010), because some studies found no evidence of HZ refuge use by
192 aquatic invertebrate fauna (Boulton et al. 2002, Olsen & Towsned 2003, James et al. 2008).
193 In contrast, the importance of the HZ as a refuge might be more evident in seasonal
194 intermittent streams. These systems are common worldwide and support diverse communities
195 of aquatic organisms including many taxa that survive in dry riverbeds and/or rapidly
196 recolonize when water returns (Stubbington & Datry 2013, Datry et al. 2014). Indeed, the
197 influence of drought may be even more intense in streams that lack a marked seasonality
198 (unpredictable intermittent streams, López–Rodríguez et al. 2012). This is the case for some
199 Mediterranean streams with supra–seasonal drought, where many organisms that survived in
200 the HZ during the dry season recolonized the stream during the first month of the wet season
201 (López–Rodríguez et al. 2012). Use of the HZ as a refuge is not exclusive to large biota, it
202 occurs across a wide range of organism size. Febria et al. (2012) observed that biofilms also
203 use hyporheic sediments as a refuge from desiccation, mainly transported by hydrological
204 pathways through the sediments. During periods of drought, the HZ supports bacteria
205 associated with the infiltration of water and the creation of microhabitat in the sediment;
206 when interstitial pore spaces become filled with water during flood events, HZ and the
207 surface become connected allowing bacteria recolonization (Febria et al 2012).

208 Another factor that markedly affects the hydrology of streams and rivers is the
209 seasonal change of in–stream vegetation cover. In–stream macrophytes are typically
210 abundant in many lotic ecosystems during spring and summer; altering river flow and
211 trapping sediments (Champion & Tanner 2000, Dodds & Biggs 2002). In–stream vegetation
212 may reach from 0% to over 70% of spatial coverage between winter and summer in European
213 rivers (Cotton et al. 2006). This increase in macrophytes is coupled with a drastic reduction

214 of the open channel flow velocity and the deposition of fine sediments (Cotton et al. 2006).
215 Nevertheless, to our knowledge, there is no published research that assesses the effect of in–
216 stream macrophyte dynamics on hyporheic flow and hyporheic communities. In addition,
217 daily fluctuations in stream flow may also be caused by evapotranspiration of vegetation
218 (including riparian vegetation) and it has been hypothesized that this transpiration enlarges
219 hyporheic flow paths during the day and decreases them at night (see Wondzell et al. 2010).

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221 Hyporheic zone as a budget of biomass and production in stream and
222 rivers

223 Defining system boundaries is an important aspect of the study of ecological processes
224 (Smock et al. 1992). Streams and rivers have been viewed traditionally as having three
225 interactive spatial compartments: open channel and benthic zone (BZ), HZ and riparian zone
226 (Cummins et al. 1983, Ward et al. 1989) and each compartment could play a different role
227 depending on the ecological process under study. Secondary production is a useful measure
228 of the energy flux (as biomass) produced by heterotrophic organisms over time and space
229 (Benke & Huryn, 2007). However, there are only a few studies which have determined the
230 relative contribution of the HZ compartment to whole system production (i.e. Smock et al.
231 1992, Collier et al. 2004, Wright–Stow et al. 2006, Reynolds & Benke 2012). Most previous
232 studies have defined the top 5–10 cm sediment layer as the benthic zone (BZ), and lower
233 depths as HZ despite its proposed biological definition (Table 1). In order to accurately
234 define the limits of the HZ compartment, a small-scale approach across a depth gradient is
235 needed.

236 Some studies have shown that invertebrate assemblages comprising a suite of
237 relatively few and large individuals near the surface are replaced by numerous but small–
238 bodied organisms with increasing depth (Schmid–Araya 1994, Stead et al. 2004). This is
239 because the hyporheos differ in their ability to utilize the HZ depending on their biological
240 attributes (Robertson & Wood 2010, Descloux et al. 2014), and their ability to penetrate into
241 the HZ (Nogaro et al. 2009). The reduction in oxygen concentration and pore size due to
242 sediment agglomeration along the depth gradient (Fig 1) limits the distribution of large
243 macroinvertebrates with higher metabolic rates (Maridet & Philippe 1995, Strayer et al.
244 1997). As a result, the density of meiofauna (microscopically small metazoans) and protists
245 should increase with depth (Fig 1). In fact, the reduction in density of large organisms
246 through depth has been broadly reported as a general pattern in studies of riverbed
247 communities (i.e. Dole–Olivier et al. 1994, Marchant, 1995, Maridet & Philippe 1995, Davy–
248 Bowker et al. 2006, Pacioglu & Robertson 2017). Accordingly, it might be hypothesized that
249 the depth gradient, as a set of different physicochemical factors, is also a key variable causing
250 the decline of biomass and secondary production of riverbed systems. Metadata analysis of
251 invertebrate communities from different river systems corroborates this prediction, showing a
252 negative and significant effect of depth both on biomass and secondary production (Fig 2,
253 Explanation of these analyses are available in appendix 1). However, despite its significance,
254 the regression model explains only a small part of the observed variation (marginal $R^2 = 11\%$,
255 and conditional $R^2 = 54\%$, see appendix 1) and so other variables must be important. This
256 meta-analysis is limited to macroinvertebrate communities because studies of changes in
257 biomass and secondary production along the depth gradient have focused exclusively on large
258 size organisms ignoring a great fraction of meiofauna and protozoa.

259 Remembering that hyporheos differ in their ability to penetrate into the HZ depending
260 on their size, we expect a significant interaction between depth and size group (flagellates,
261 ciliates, meiofauna and macroinvertebrates). Including these groups and their interaction with
262 depth, hydrology and sediment characteristics in future research studies would notably
263 improve predictive modelling and compartment comparisons. This is of particular interest
264 because it has been proposed that the bioreactor ability of HZ is sustained and maintained by
265 diverse and active hyporheic communities (Krause et al. 2009). Accordingly, it might be
266 predicted that hot spots of nutrient and pollutant transformation may coincide with areas
267 containing higher biomass and secondary production rates. It could also be expected that the
268 role of different organisms in the bioreactor capacity would vary following the depth
269 gradient. For example, bioturbation and bioirrigation resulting from life activities of
270 relatively large burrowers (such as Chironomidae larvae, Ephemeridae nymphs or
271 Oligochaeta), would be more important in the benthic zone and upper layers of the HZ than
272 in deeper levels. These processes promote sediment permeability, respiration of freshwater
273 sediments and bacterial activity (Betrics and Ziebis 2009, Hölker et al. 2015, Baranov et al.
274 2016), and so have a great impact on water biogeochemistry (Morad et al. 2010).

275

276 ## The hyporheic bioreactor

277 Flow exchange and pore water chemistry of HZ can be also affected by anthropogenic
278 activity, typically with negative effects on ecosystem health. A common alteration that occurs
279 in rivers across the world is the artificial water input from Wastewater Treatment Plants
280 (Carey & Migliaccio 2009) where many rivers undergo daily inputs of nutrient (i.e. organic

281 carbon, nitrate, phosphate) and other pollutants (i.e. pesticides) as a result of human activities
282 (Boyer et al. 2006, Mulholland et al. 2008). However, once these compounds penetrate into
283 the sediments as a consequence of the hydrological patterns, they may be transformed into
284 oxidized or reduced substances by metabolic reactions, mediated by active and productive
285 hyparchic communities (Krause et al. 2009, Bardini et al. 2012, Sánchez–Perez et al. 2013).

286 Accordingly, the HZ acts as a true bioreactor with water purifying ability and
287 microbial biofilms play an important role. Hyporheic biofilms are dominated by highly
288 diverse bacteria and archaea communities embedded in the same matrix of polysaccharides
289 (Batin et al. 2016). This results in the coexistence of a great range of operational taxonomic
290 units (Zeglin 2015), diverse metabolic capabilities (Singer et al. 2010, Battin et al. 2016) and
291 sites of high enzymatic activity (Romaní et al. 2008). Thus, in stream biofilms are also
292 important components of the global biochemical fluxes of carbon, nitrogen and phosphorous
293 (Mulholland et al. 2008, Battin et al. 2008, Boano et al. 2014). The supply of nutrients is
294 assumed to be a limiting factor in determining the biomass, activity, and physiology of
295 subsurface microbial communities (Bengtsson 1989). Thus bacterial biomass and metabolic
296 activity should be significantly greater under situations of higher input of DOC (Foulquier et
297 al. 2011). In addition, water is pumped in and out of the HZ and riparian zones on a daily
298 cycle because water stage variation, generates large hydraulic gradients and enhanced mixing
299 in highly regulated rivers (Gerecht et al. 2011). These daily fluctuations of river–stage
300 stimulate bacterial respiration and organic carbon turnover (Stegen et al. 2016).

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302 ## Micropollutants, the new challenge for the hyporheic bioreactor

303 Recent research has shown that nitrogen, phosphorus and organic carbon are
304 important pollutants in the HZ and aquatic ecosystems generally (i.e. Lewandowski &
305 Nützmann, 2010, Bardini et al. 2012, Harvey et al. 2013, Maazouzi et al. 2013). However,
306 surface water systems and their interactions with groundwater systems are increasingly under
307 pressure from a new group of chemicals; the micropollutants (Langenhoff et al. 2013). The
308 occurrence of micropollutants (such as pharmaceutical and personal care products; i.e.
309 ibuprofen or antibiotics) due to WWTP inputs has greatly increased in stream and rivers. The
310 concern about their presence is mainly related to potential adverse effects on environmental
311 systems (i.e. bioaccumulation) and to human toxicology (i.e. aquifer contamination)
312 (Hernández Leal et al. 2010, Langenhoff et al. 2013). Furthermore, the chronic low–level
313 antibiotic exposures detected in aquatic systems acts as a selective process on bacteria
314 communities (Hirsch et al. 1999, Yang & Carlson 2003). Thus, differential
315 antibiotic tolerance of a bacterial community may produce a shift in the biofilms structure
316 (composition, richness, density), affect the spatial distribution of members of the community
317 (Roose–Amsaleg & Laverman 2016) and change the ability of biofilms to conduct ecosystem
318 services (e.g. reducing denitrification processes due to deleterious impacts on denitrifying
319 bacteria, Costanzo et al. 2005). However, in some cases, micropollutants can be efficiently
320 attenuated along flow paths into the HZ (Lewandowski et al. 2011). Indeed, some of these
321 compounds (i.e. diclofenac, bezafibrate, ibuprofen, and naproxen) are more efficiently
322 transformed in river sediments than in wastewater treatment plants (WWTP) by biofilms (i.e.
323 Schulz et al. 2008, Radke et al. 2009). This is mainly related to the higher diversity of
324 microbial communities in environmental systems. In addition, water residence times in the

HZ are longer than in the open channel and surface sediments (and WWTP), allowing more efficient biodegradation processes (Lewandowski et al. 2011).

Notwithstanding the role of the biofilm is recognized in pollutant attenuation, more complex questions behind this ecological process remained unanswered (i.e. the role of the rest of the community). Furthermore, it is also important to consider the hierarchical interaction between hydrological patterns and biochemical processes in the study of the nutrient and pollutant breakdown into the HZ. Hydrodynamics throughout the sediments may induce two opposite effects on solute reactions. Higher inward water fluxes lead to a larger input of substances into the HZ enhancing reaction rates, but also the hyporheic microbiota has less time to perform biochemical reactions due to the lower residence time of the compounds in the sediments (Fig 3a) (Bardini et al. 2012). These mechanisms will become even more complex when we recall that hydraulic conductivity may also be affected by the action of the hyporheos. Growth of biofilm matrices in the sediment pores reduces permeability and increases residence times of water into the HZ (Findlay & Sobczac, 2000, Battin et al. 2003). In addition, biofilm theory holds that uptake of solutes is diffusion limited by the thickness of the biofilm polysaccharide matrix (Gantzer 1988). Before assimilation, solutes must pass first from the pore water to the biofilm surface (external mass transfer) and then through the biofilm matrix to the cells (internal mass transfer) (Battin et al. 2003). However, sediments are constantly being reworked, increasing the sediments permeability locally (Boulton, 2000). Life activities of macroinvertebrates, meiofauna and protists (e.g. ciliates and flagellates) in the HZ (digging, removing the sediments and grazing on biofilms) result in preferential flow paths, increasing biofilm surface and boosting bacterial densities (Danielopol 1976, Boulton 2000, Mermillod–Blondin et al. 2003, Battin et al. 2003), acting

as ecosystem engineers (Fig 3c, d). Thus, the net effect on breakdown rates depends on the balance between all these opposing factors (Arnon et al. 2007, Cardenas et al. 2008, Bardini et al. 2012). In this manner, mechanistic understanding of biofilms function can be acquired only through carefully designed experiments under well–defined conditions and appropriate cultivation techniques (Singer et al. 2006). Accordingly, controlled experiments are needed to explore the underlying causal mechanisms that generate the patterns seen in reach–scale descriptive surveys (Boulton et al. 1998, Olsen and Townsend 2003).

Concluding remarks

Ecological research in the HZ is still a challenge due to the great number of variables involved in its operation. Furthermore, there is a notable hierarchy of these variables, many of them being nested or correlated spatially and temporally. Its study is also becoming one of the most developed areas in freshwater science in recent years, principally due to the development and increasing accessibility of better technological approaches. Nevertheless, studies on the role of the HZ in the functioning and ecosystem service delivery of the whole river system are still maturing and remain a major research focus and challenge for future freshwater researchers (Robertson and Wood 2010). Complex and interesting questions about how pollutants and nutrients are transformed by the hyporheos during their travel throughout the HZ, or how hyporheic hydrodynamics may determine energy fluxes of these communities are still unanswered. Furthermore, future research should also consider and focus on a more thorough understanding of impacts caused by rivers regulation on hyporheic hydrodynamics (Gerecht et al. 2011). Accordingly, holistic and truly interdisciplinary approaches at the

interface of hydrology, geomorphology and ecology will be the only valid strategy to assess all these issues (Pacioglu 2010, Boulton et al. 2010, Robertson and Wood 2010).

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Appendix

Biomass and secondary production data along depth gradient were extracted from reported values in Smock et al. (1992) and Reynolds and Benke (2012). A similar sampling method (sediment corers) and spatial scale resolution were used in both studies. Reynolds and Benke (2012) reported biomass and secondary production of the whole community of macroinvertebrates in a sandy–sediment river (Buzzards Branch). While, Reynolds and Benke (2012) measured genus–specific biomass and secondary production of chironomid larvae (Diptera) assemblage (composed by 26–31 genera) along a hyporheic gradient, comparing gravel–cobble of a high–alkalinity stream (Hendrik Mill Brach) with sandy and mud–silt habitats of a low–alkalinity stream (Payne Creek). Those chironomid assemblages were reasonably similar between studied streams. Furthermore, chironomid larvae are usually the dominant and richest invertebrate group in freshwater benthic habitats (Ferrington et al. 2008). Hence, it could be assumed as a good model group to infer general production patterns of invertebrates.

Two linear mixed effect models (LMMs) were applied to test the effect of, depth (continuous covariant) on biomass and production (responses). Responses were Log_{10} transformed to solve heterogeneity in the residuals. An Applicable Information Criterion (AIC) was then used to find the most parsimonious model by combining the fixed term (depth) and potential random effects (i.e. studied site, differences between taxa). As result, biomass and production models included depth (single covariate), study site (studied river as random intercept) and the interaction between depth and taxa (random slope) as effective parameters:

$$Log_{10}(\hat{y}_{ij}) = \beta_0 + a_{site} + \beta_1 \times Depth_j + b_i \times Depth_j + \varepsilon_{ij}$$

$$\varepsilon_{ij} \sim Norm(0, \sigma^2)$$

$$a_{site} \sim Norm(0, \sigma^2_{site})$$

$$b_i \sim Norm(0, \sigma_i^2)$$

where $\hat{y}_{ij}$ is the biomas or production for each taxa *i* at depth *j* (0,10…,50). Intercept of the model is given by $\beta_0 + a_{site}$ with changes randomly by a_{site}, and $b_i \times Depth_j$ represent the random variation of slope β_1.

Model validation was applied following Zuur et al. (2009). Previous models were fitted using the restricted maximum likelihood estimation (REML) with functions *lmer* of the R package lme4 (Boulton et al. 2016, R Core Team 2016). Finally, 5000 values from the posterior joint distribution of the model parameters were simulated with the function *sim* of the R package arm (Gelman and Hill 2007). This function uses an analytical direct–simulation method with uninformative priors (Korner–Nievergelt 2015). Obtained means of the simulated values from the joint posterior distribution of model parameters were used as

411 estimates, and the 2.5% and 97.5% quintiles as lower and upper limits of 95% credible
412 intervals. Finally, the marginal and conditional R^2 (as a technique to describe the predictive
413 capacity of mixed effect models; Nakagawa & Schielzeth 2013) was calculated to assess
414 model fit.

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808 **Legend Figures and Tables**

809 **Table 1.** Glossary of terms.

810 **Fig 1.** Scheme of the streambed community structure throughout the depth profile in relation
811 to pore size and redox potential. Arrows represent colonization depth of large
812 macroinvertebrates (a), temporary and permanent meiofauna (b) and protozoa (c). Also
813 shown is the theoretical boundary between benthic zone (BZ) and hyporheic zone (HZ) as the
814 colonization limit between benthos and hyporheos. Organisms are not drawn to scale.

815 **Fig 2.** Depth–related biomass and production of invertebrates based on reported values from
816 Smock et al. (1992) and Reynolds Jr. and Benke (2012). Predictions (black line) represent the
817 $\log_{10}$ biomass and production values and are derived from the linear mixed models explained
818 in the Appendix, with 95% credible intervals (shaded grey). Open circles represent the $\log_{10}$–
819 transformed values per taxa.

820 Fig 3. The ability of the HZ to process dissolved solutes is mediated by a hierarchical
821 interaction between hydrological patterns and community ecology. (**a**) Daily and seasonal
822 fluctuations between the high river–stage (H–RS) and Low river–stage (L–RS) cause
823 variation in the open channel discharge. (**b**) The increase in the surface flow promotes higher
824 inward water fluxes (sub–surface flow) and input of dissolved solutes into the HZ, but also
825 the residence time of water in the sediments decreases. (**c**) Life activities of
826 macroinvertebrates and meiofauna result in bioturbation and biorrigation phenomena in the
827 streambed sediments, causing the occurrence of preferential flow paths and increasing
828 permeability locally. (**d**) Protists grazing on biofilms increase its absorption surface. As a

result, dissolved solutes diffusion gradient is higher in presence of grazers (**d1**) than in their absence (**d2**).

846 TABLE 1

Term	**Definition**	**Source**
Hyporheic zone (Original definition)	The interstitial habitat beneath a stream, bordered by the surface water above and by the true groundwater below	Orghidan (1959)
Hyporheic zone (Geochemical definition)	The volume of sediment containing a specified percentage of surface water	Tonina and Buffington (2009)
Hyporheic zone (Hydrological definition)	The volume of sediment where water interchange between open channel and groundwater occur as a result of streambed pressure gradients and hydraulic conductivity	Tonina and Buffington (2009)
Hyporheic zone (Biological definition)	The volume of sediments housing a characteristic hyporheic community. This community can be defined as occasional users or permanent users	Tonina and Buffington (2009)
Hyporheic zone (Integrative definition)	Any location meeting four key criteria: [1] Saturate surface. [2] Existence of flow path that originate from and return to surface water. [3] Interaction with the stream occurs within a temporal scale relevant to the	Ward (2016).

	processes of interest. [4] Processes of interest occur continuously from subsurface to the groundwater continuum.	
Hyporheos	The biota occupying saturated interstitial spaces below the stream surface (benthic zone)	Standley & Boulton (1993)
Up–welling (UW) zone	Low–pressure areas in riverbed, where surface water comes out from HZ to the open channel	Franken et al. (2001)
Downwelling (DW) zone	High–pressure areas in riverbed, where surface water enter into the HZ	Franken et al. (2001)
Hyporheic hydrodynamics	Strength and direction of the water mass through the sediment pore spaces in the HZ, resulting from the alternation of UW and DW zones	This article
Micropollutants	A vast and expanding array of emerging contaminants (including pharmaceuticals, personal care products, steroid hormones, industrial chemicals and pesticides) commonly present in waters at trace concentrations, ranging from a few	Luo et al. (2014)

	ng/L to several μg/L.	
Hyporheic biorreactor	Active biological system in which the transformation of chemical compounds occurs as result of the hyporheos life activities or the active substances they produce.	This article
Residence time	Hydrodynamic retention time during the hyporheic exchange during which biogeochemical processesing of disolved solutes occur in the HZ.	This article

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FIG 1

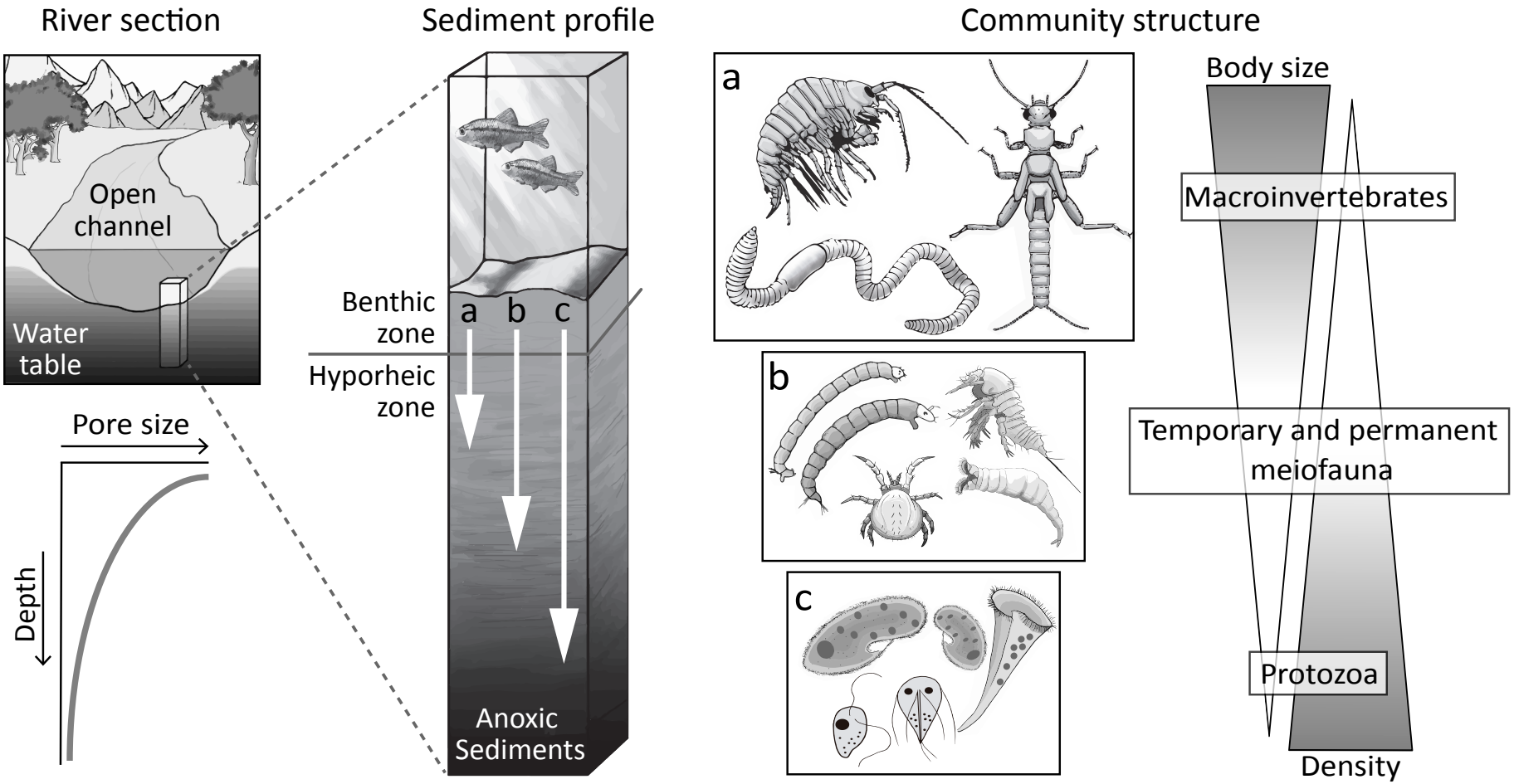

River section
Sediment profile
Community structure
Open channel
Water table
Pore size
Depth
Benthic zone
Hyporheic zone
a
b
c
Anoxic Sediments
a
b
c
Body size
Macroinvertebrates
Temporary and permanent meiofauna
Protozoa
Density

FIG 2

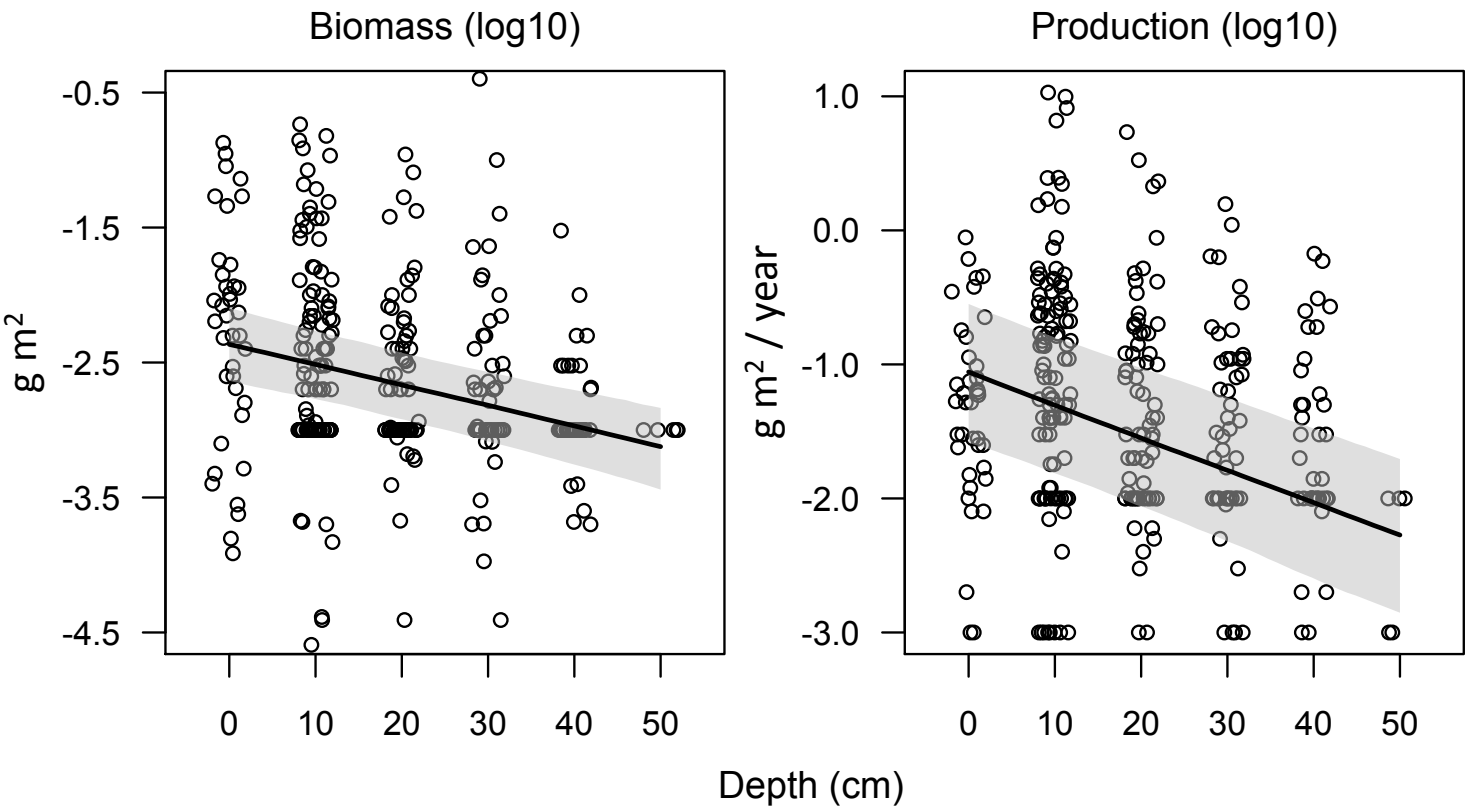
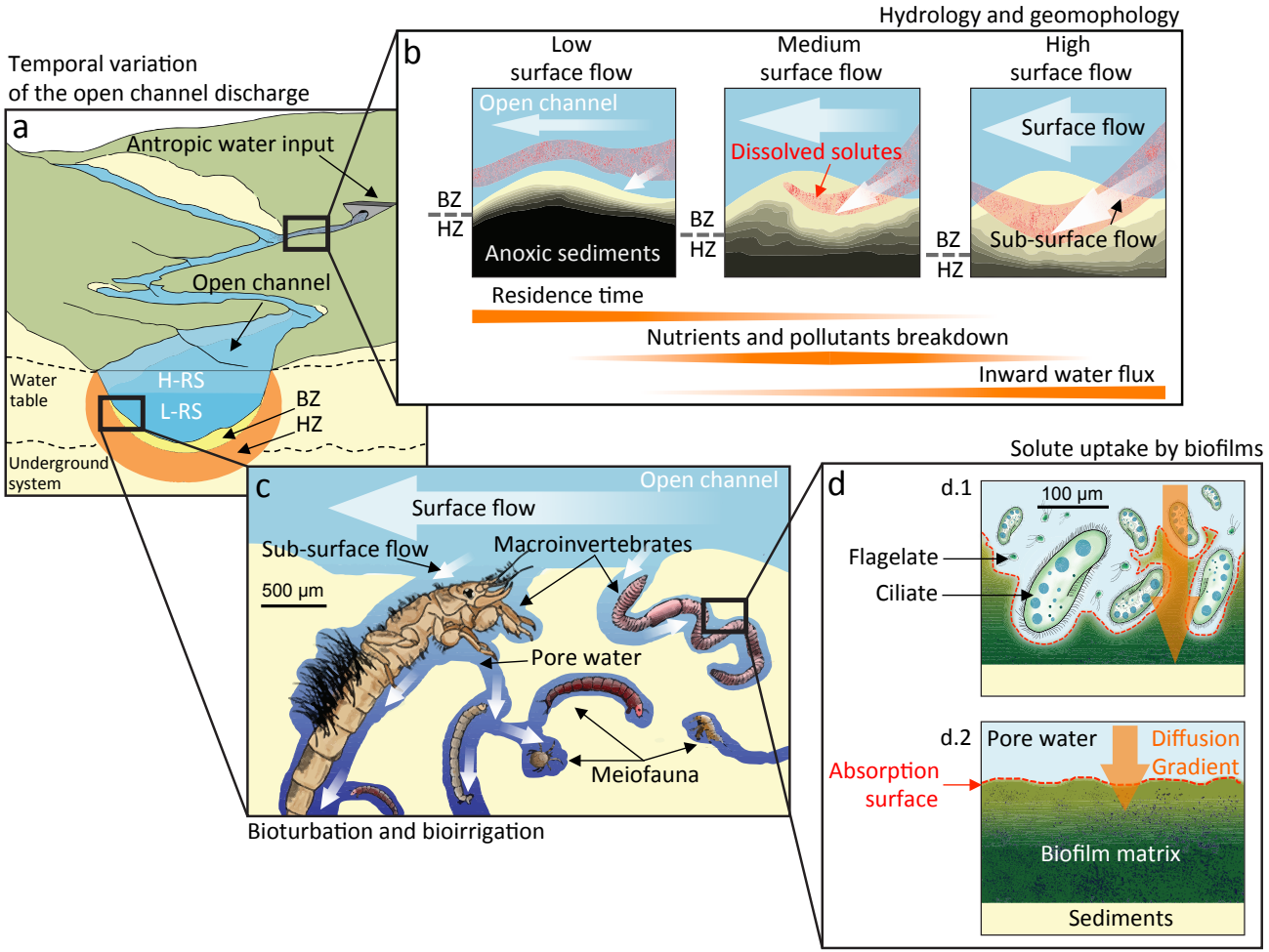

Biomass (log10)
Production (log10)
g m²
g m² / year
-0.5
-1.5
-2.5
-3.5
-4.5
1.0
0.0
-1.0
-2.0
-3.0
0
10
20
30
40
50
Depth (cm)

FIG 3


Temporal variation
of the open channel discharge
a
Antropic water input
Open channel
Water
table
H-RS
L-RS
BZ
HZ
Underground
system
Hydrology and geomophology
b
Low
surface flow
Medium
surface flow
High
surface flow
Open channel
Dissolved solutes
Surface flow
BZ
HZ
Anoxic sediments
Sub-surface flow
Residence time
Nutrients and pollutants breakdown
Inward water flux
c
Open channel
Surface flow
Sub-surface flow
Macroinvertebrates
500 µm
Pore water
Meiofauna
Bioturbation and bioirrigation
Solute uptake by biofilms
d
d.1
100 µm
Flagelate
Ciliate
d.2
Pore water
Diffusion
Gradient
Absorption
surface
Biofilm matrix
Sediments